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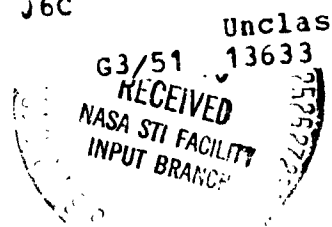
MICROORGANISMS IN THE STRATOSPHERE

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Information has been published on the lower limits of the biosphere, since life has been discovered in earth sampled from different oceanic troughs at a depth of 10-11 km. It is much more difficult to establish the upper limits of the biosphere due to procedural difficulties.

Saprophytic fungi and sporiferous bacteria were discovered in a sample taken at an altitude of 20 km by a cylindrical sampling device raised in 1936 by an Explorer II stratospheric balloon and lowered by parachute, even though both the sampling device and the parachute had been sterilized beforehand. Various microorganisms were discovered with sampling devices raised in 1962-1964 by a stratospheric balloon filled with helium. However, the latter data cannot have scientific significance because the skin of the stratospheric balloon was not sterile. At the time of its filling with helium it lay on an oat field, and during its flight the sampling devices drew in a large quantity of diverse soil microorganisms released by the balloon's skin (2). This exhausts information on presence of microorganisms at high altitudes.

There is great interest in establishing the upper limit of the biosphere because the data obtained would permit us to assess the altitude to which microorganisms can rise in the stratosphere, their stability against extreme physical factors, the quantitative and qualitative composition of microflora of the troposphere and stratosphere, and so on.

In procedural respects, analysis of the stratosphere is rather complex, since the possibility that foreign microflora may enter the analyzer's sampling device must be excluded completely when samples are taken. An analyzer carried by a meteorological rocket was designed and manufactured according to technical specifications provided by microbiologists. The apparatus was described in detail by experts in instrument making.

In our research we used the following procedures for microscopic analysis of the stratosphere.

1. Meteorological rockets were launched to an altitude of up to 100 km to take air samples. During the time the rocket passed through the dense layers of the atmosphere its nose was heated to temperatures insuring death of all microorganisms. The rocket's nosecone, which housed the instrument

compartment, was jettisoned at the required altitude by explosive charges. Samples were taken only in the stratosphere as the rocket ascended, completely excluding the possibility that foreign microorganisms would enter the sample. The rocket's top section and jettisonable nosecone are shown in Figure 1a.

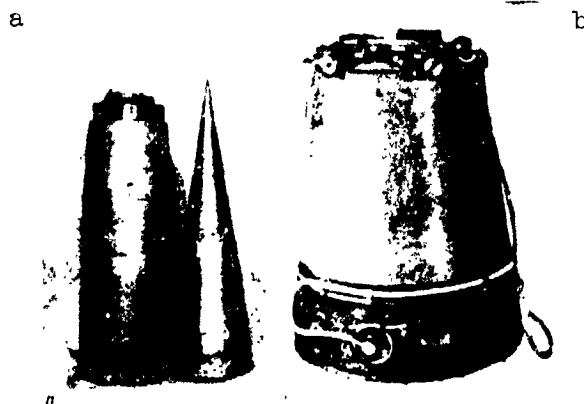


Figure 1. Top Section of the Rocket and the Jettisonable Nosecone (a), and the Sampling Analyzer (b). The moving sticky tape on which particles settle as the rocket ascends can be seen.

After the nosecone is blasted away the ascending rocket is topped by the analyzer cover, which is subsequently deployed by two explosive charges. A device containing nutrient medium was present in the analyzer. Deployment of the sampling device permitted settling of all aerosol particles, including microorganisms, on the nutrient medium. After the samples were taken the rocket's instrument compartment was returned to the ground by parachute. The analyzer is shown in Figure 1b.

3. Prior to being placed in the analyzer, the device was sterilized without nutrient medium in an autoclave at 0.5 atm for 30 minutes, after which it was filled with nutrient medium by a procedure insuring sterility. Together with the nutrient medium the analyzer was inserted successively into two polyethylene bags, which were sealed and then installed in commercial containers. The latter were subsequently treated by  $\gamma$ -rays (at a dose of 3.2-3.5 millirads, a dose intensity of 98.8 rads/sec, and a radiation treatment time of 9 hours). Such sterilization did not reflect upon either the analyzer's functional properties or on the nutrient medium's quality. The sealing of the device and its nutrient medium were tested after sterilization by different methods. To establish the  $\gamma$ -ray sterilization conditions we employed radioresistant spores in 14 spots within the analyzer. Death of all test spores confirmed the effectiveness of sterilizing the analyzers with radiation.

4. The devices containing nutrient medium, present in the stratosphere together with analyzers, were inserted into sterile commercial cassettes and kept in a thermostat at 30° for 7-14 days, after which time they were examined under a microscope at low magnification. The time of appearance and the nature of colonies that grew were recorded. Only colonies grown from cells entering the analyzer during the sampling procedure as the rocket ascended were counted. Then the device containing nutrient medium was opened, and microorganisms from the colonies that grew were seeded in appropriate nutrient mediums in test tubes. Later the isolated microorganisms were studied.

Results. In all, six rockets carrying analyzers were launched. Despite jettisoning of nosecones, the analyzers failed to operate in two rockets. In one case not a single microbe settled on the surface of the nutrient medium after samples were taken and, correspondingly, no colonies were found. This circumstance confirms the reliability of apparatus sterilization. In the three remaining cases microorganisms settled on the nutrient medium, and colony growth was recorded.

The results of microbiological analyses in the stratosphere are shown in Table 1.

Table 1

Rocket Number	Altitude at Time of Sampling, km	Total Number of Grown Colonies
1	48-58	20
2	61-70	3
3	61-75	-*
4	57-77	8
	77-85	0

\*No colony growth was observed.

Research demonstrated that among colonies of microorganisms, the microscopic fungi *Circinella muscae* (Figure 2a), *Penicillium*, *Aspergillus niger*, and *Mycelia sterilia* were found. In addition we detected a culture of microbacteria and micrococcus (Figure 2b), forming yellow-orange and white colonies respectively on dense nutrient mediums. Isolated microorganisms were subjected to thorough study. A significant point is that the colonies developed within the center of the analyzer's nutrient medium, and not at the edge, as would have occurred in the event of microbial contamination. As we know, fine mineral particles rise from the earth's surface and can reach high altitudes. In this connection a comparison of the weight of mineral particles with the weight of dried fungal spores and

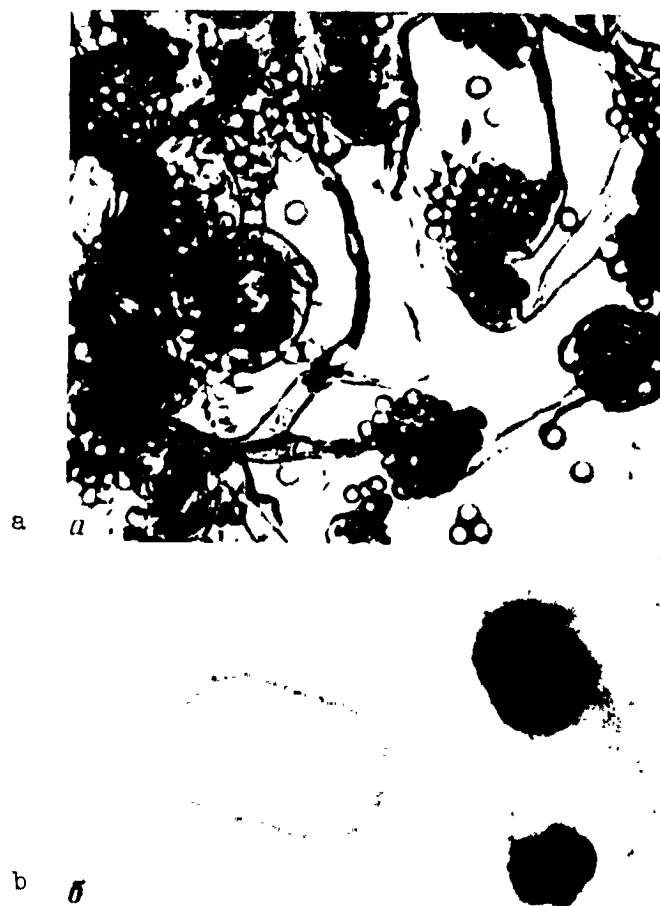


Figure 2. *Circinella muscae* Mycellium Spores (a) and Dividing Micrococcal Cells (b) Isolated From the Stratosphere: a--500 $\times$ , b--12,625 $\times$ .

conidia or bacterial cells isolated from the stratosphere would be of doubtless interest. We cannot reject the possibility that fragments of fungal mycellium, capable of faster germination than fungal conidia or spores, may ascend. This is confirmed in particular by detection of a sterile mycellium in the stratosphere, which subsequently began to grow.

The data presented above should be interpreted as a preliminary communication and, naturally, more rockets carrying analytical apparatus will have to be launched into the ionosphere, with sampling beginning in the central layers of the stratosphere.

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